Cell Growth and Size Homeostasis in Proliferating Animal Cells

Amit Tzur, Ran Kafri, Valerie LeBleu, Galit Lahav, Marc W. Kirschner

Analiese DiConti

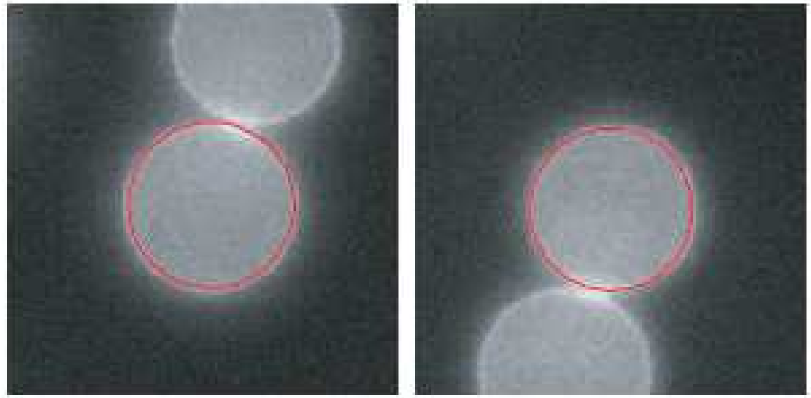
November 19, 2009

Experimental Question

- Is there an intrinsic mechanism for coordinating growth and cell cycle in metazoan cells?

- If so, what is this mechanism dependent on?

Background



- Two models:
  - Exponential growth
  - Linear growth
- Other systems
  - Budding Yeast – size dependent growth rate
  - Bacteria - unknown

Background

- Metazoan System
  - Unclear whether regulation exists
  - Regulation may be the result of separate growth and mitogenic signals from the environment
  - Conflicting studies
- Statistics
  - Need for synchronized populations
  - Cell Cycle Inhibitors

Background

- Purpose: Determination of the growth function for lymphoblastoid lukemia cells

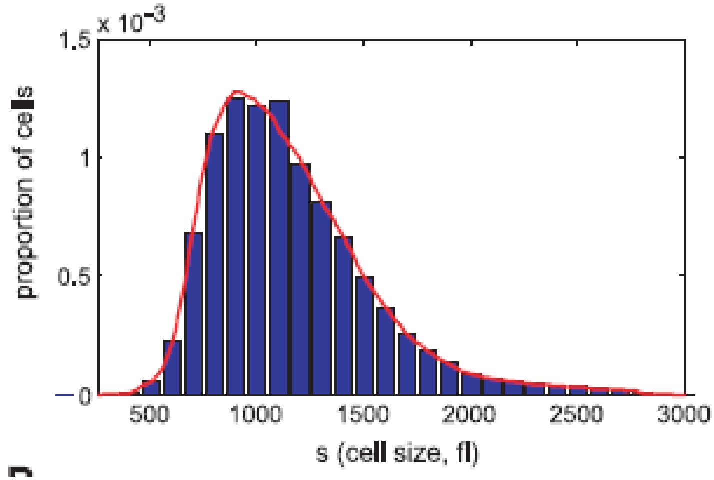
- How: Combined a gentle cell synchronization technique with mathematical analysis

Measuring size dependency on growth in asynchronous populations

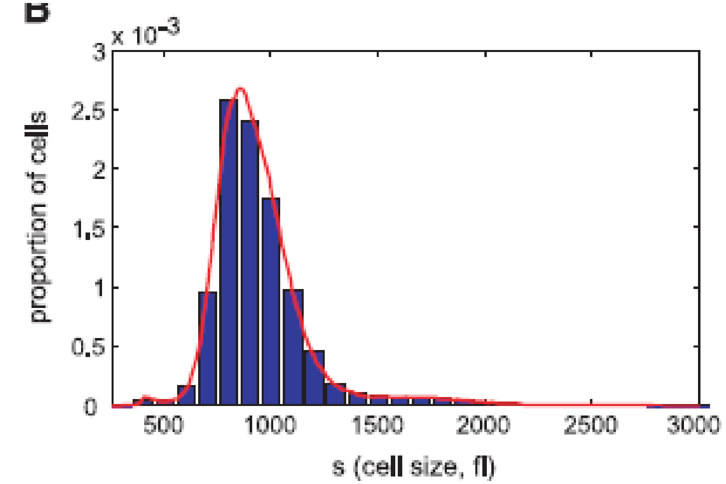
- Collin Richmond Method:
  - At steady state, # cells smaller than size S only increases when cells larger than size S divide and only decreases when cells smaller than size S grow
  - Proportion of cells of any size does not change in time, so two numbers must be equal
- Problem: Requires size distribution of newborn subpopulation and distribution of cells just before division. Difficult to obtain

Measuring size dependency on growth in asynchronous populations

- Subpopulation of newborns
  - Mouse lymphoblastoids on a membrane
  - Division
    - One daughter cell eluted
    - Other continues to divide

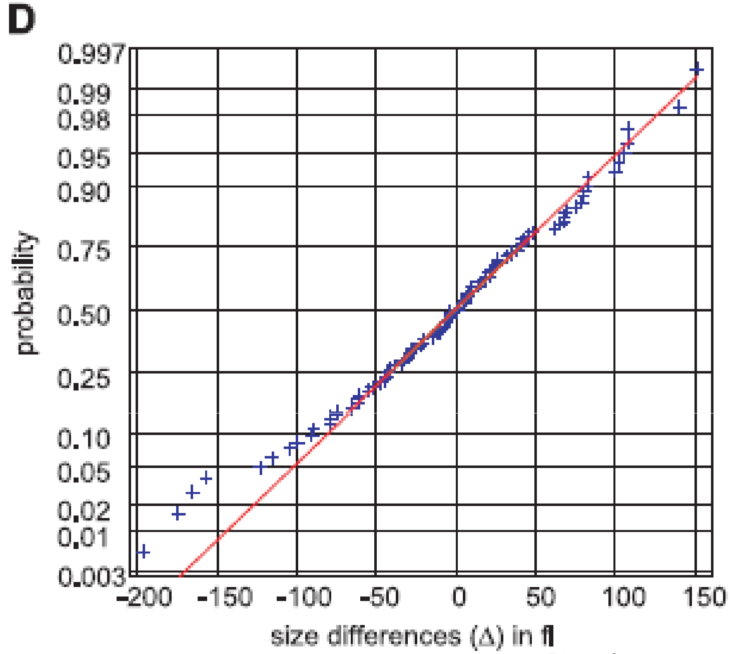


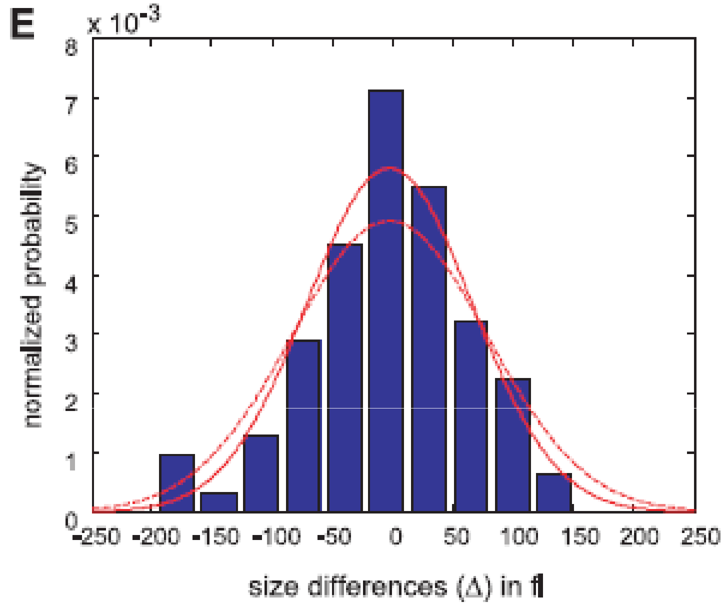
Unsynchronized Population

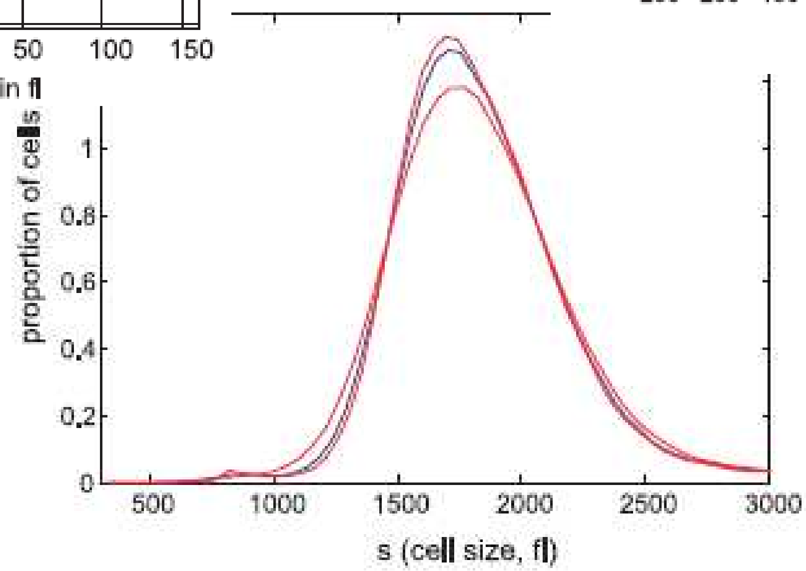


Newborn Population

Measuring size dependency on growth in asynchronous populations



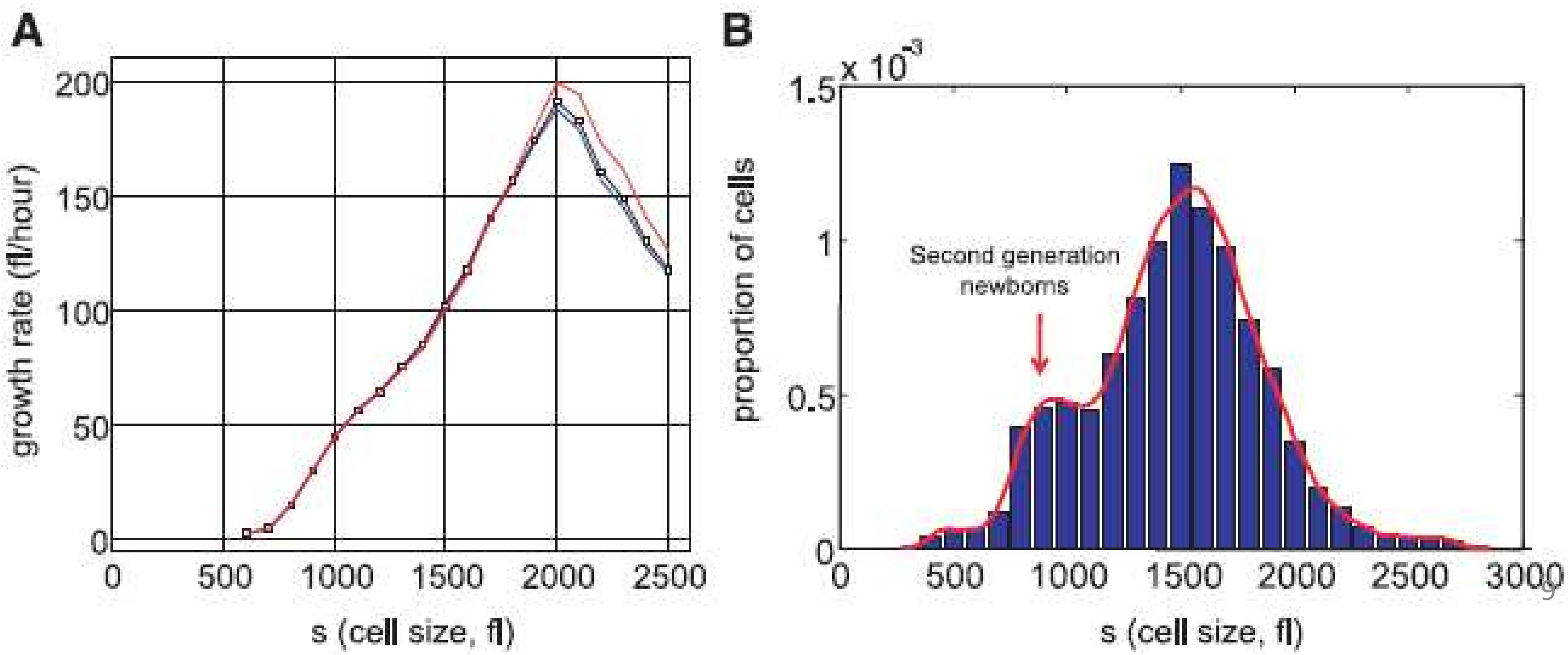




Implementation of Collins-Richmond Method

$$\upsilon(s) = 2\alpha\frac{F_0(s)}{f_a(s)} - \alpha\frac{(F_0 * \delta)(s)}{f_a(s)} - \alpha\frac{F_a(s)}{f_a(s)}$$

v (s)= Cell Growth Rate
$f_a(s)$ = asynchronous size probability distribution
$F_a(s)$ = cumulative $f_a(s)$
$F_0(s)$ =newborn cumulative size distribution
$\delta(\Delta)$ = distribution of differences between newborns
α = fraction of dividing cells/minute



Conclusions from Collins-Richmond

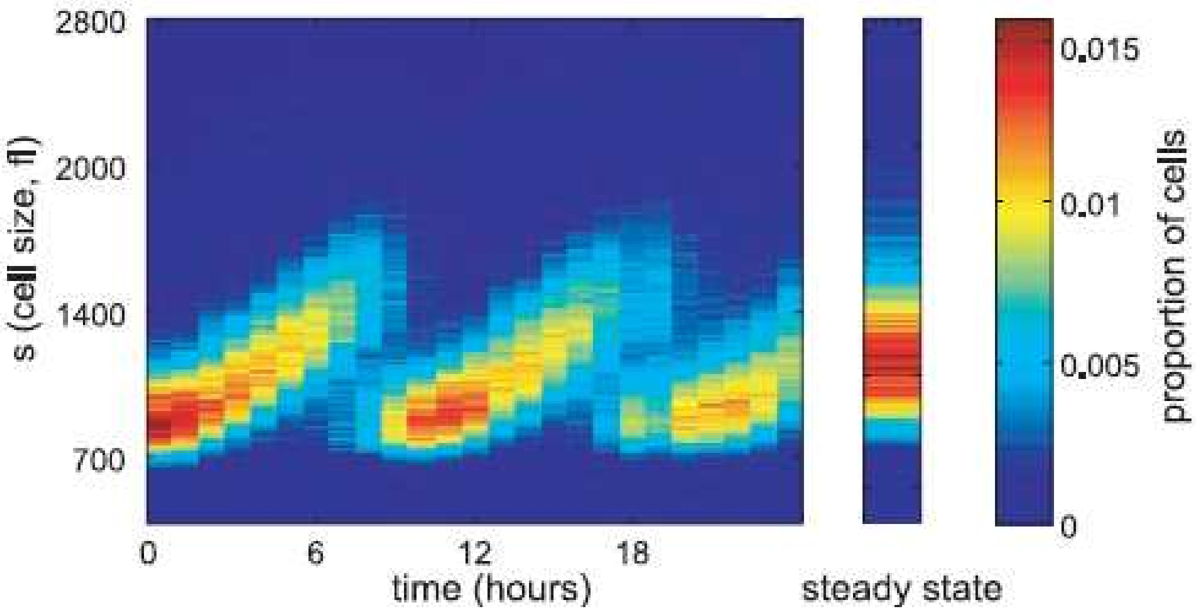
- Larger cells have higher growth rates throughout most of cell size range.
- Beyond critical size (vol = 2000fl for L1210), trend is reversed, but 65% of population has already divided before reaching this size

But, C-R is inadequate because of growth rate heterogeneity within the population

Time dependency of growth

- Comparison of pairs of size distributions from synchronized populations at one hour intervals
- Average rate at which cells grow in each interval

$$\beta_n = \frac{1}{N_t}\sum_{i=1}^{N_t}\beta_n^i$$



Time dependency of growth

$$f_n(s) = \int_{c=0}^{\infty} f_0(s-c)\varphi_n(c)dc$$

Deconvolve to find $\varphi_n(c)$ then use that to find c_n for each time point

$$c_n = \int c\varphi_n(c)dc = \langle c_j^i \rangle$$

Relate calculate values c_n to the mean growth rates β_n

$$\beta_n = c_n - \sum_{j=0}^{n-1} \beta_j \Delta t$$

Because $\Delta t = 1$,

$$\beta_n = c_n - \sum_{j=0}^{n-1} \beta_j$$

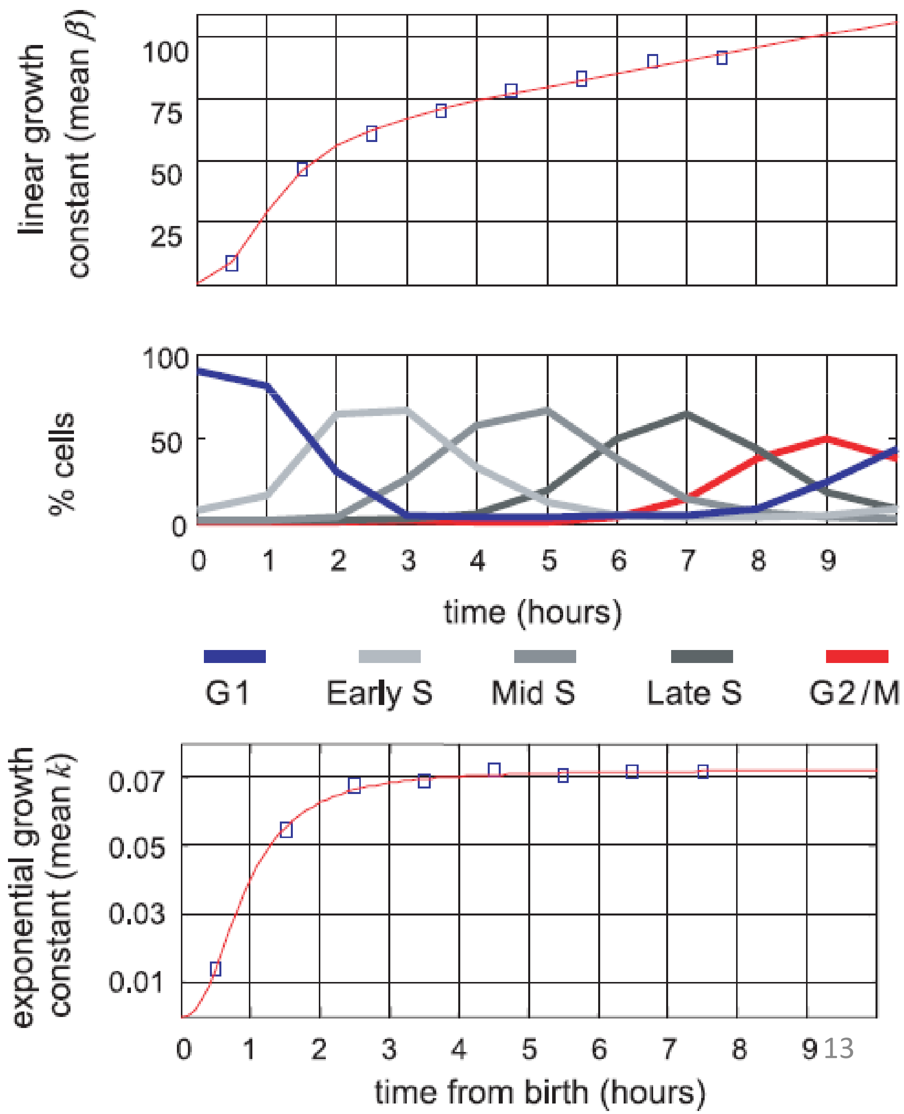
c_n = mean c value at t=n

$\varphi_n(c)$ = probability distribution of c^i_n

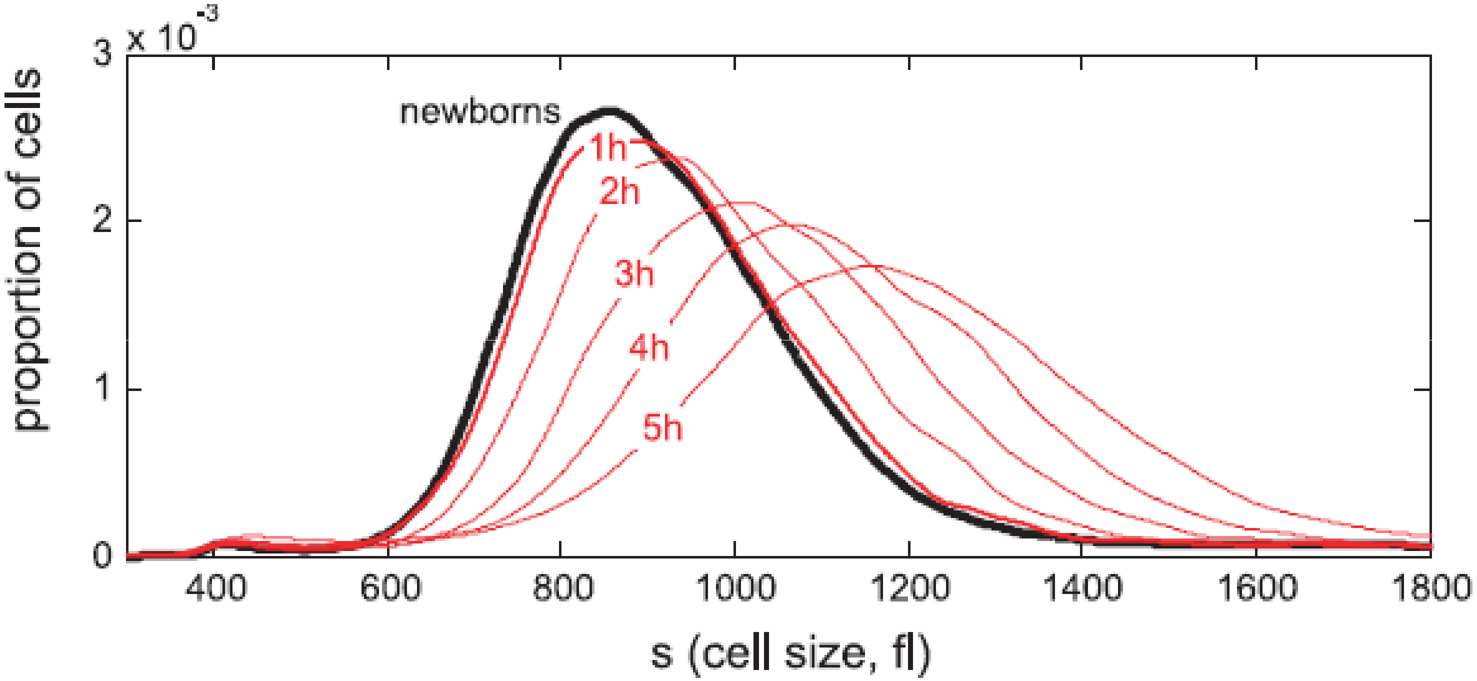
f_n = measured distribution from time n

Time dependency of growth

- Mean linear growth constants for each time interval
- Distribution of cell cycle stages
- Mean exponential growth constants for each time interval



Time dependency of growth

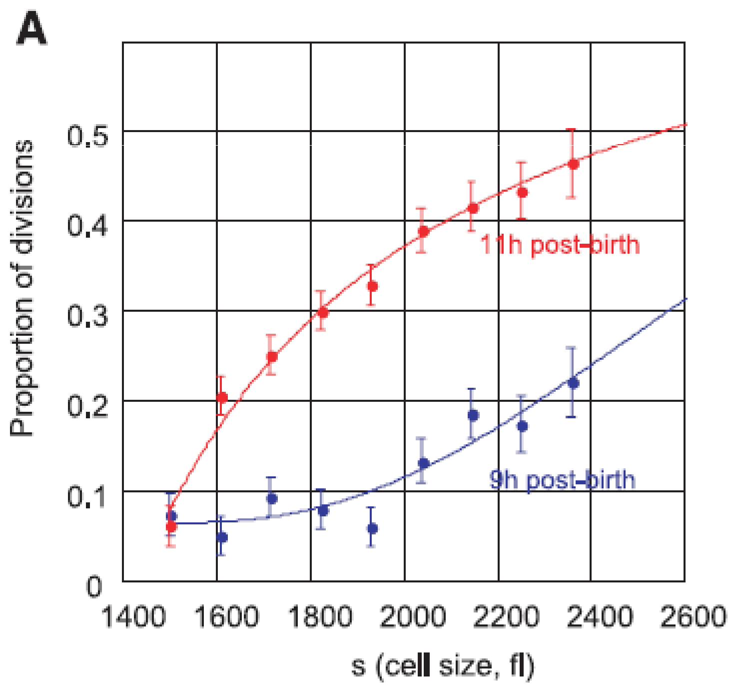


Dependence of Cell Division on Time and Size

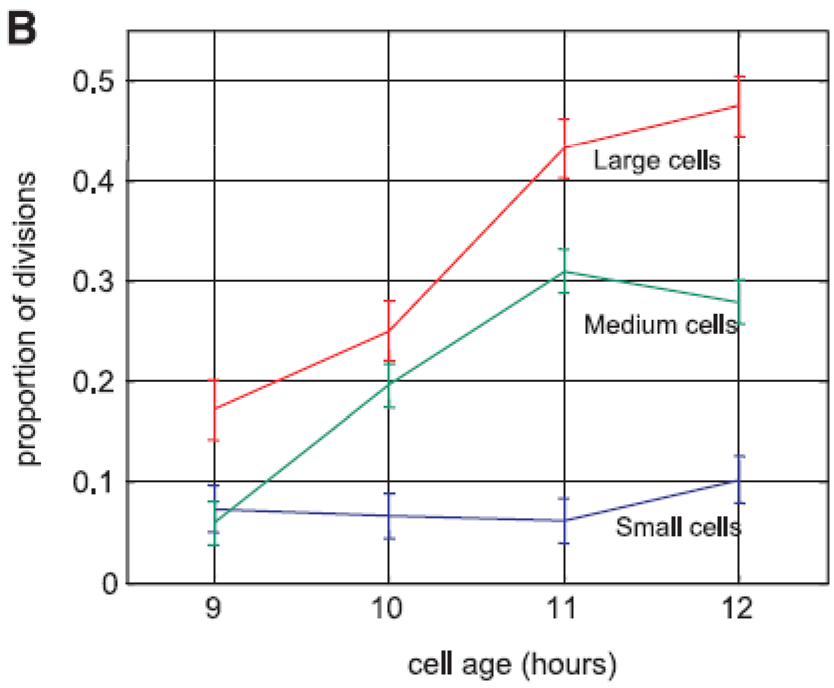
- Is there a size gate that shortens the cell cycle?
  - Examine Interval at which most cells divide (9-12 hours)
  - Use growth exponential constants and compare size distributions at consecutive time intervals

Dependence of Cell Division on Time and Size

- Calculate frequency of cell division as a function of cell cycle time



Cells at same age



Cells at same size

Dependence of Cell Division on Time and Size

- If cells were all same age, likelihood of division increased with increasing cell size
- If cells were all same size, likelihood of division increased with increasing age
- If ψ = likelihood of division, τ= cell age, and s = cell size, probability of division:

$$d\psi = \left(\frac{\partial \psi}{\partial \tau}\right)_s d\tau + \left(\frac{\partial \psi}{\partial s}\right)_\tau ds$$

Discussion

- Size of cell reflects the relationship of its growth rate and division frequency
- Accelerative growth phase in G_1 follow by stable exponential growth during the rest of the cell cycle
- Growth and division independently determined by size and age
- Very large cells (above threshold) behave differently than this model